0022-202X/81/7702-0250$02.00/0
THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 77:250–251, 1981

Vol. 77, No. 2
Printed in U.S.A.

LETTERS TO THE EDITOR

We are pleased to receive Letters to the Editor on appropriate subjects. These letters should be submitted in typewritten form, double-spaced, and are not to exceed 2½ pages. When appropriate, we will solicit comments from the original authors. All Letters to the Editor are subject to editing and possible abridgment.

Fd DEFICIENCY AND MOLECULAR SIZE OF SCLEROMYXEDEMA MONOCLONAL IMMUNOGLOBULINS

To the Editor:

Recently [1], Fd deficiency of the monoclonal IgG-lambda immunoglobulin has been observed in a patient with scleromyxedema. The molecular weight of this paraprotein was 110,000 daltons. This stimulated us to perform similar investigations in 2 cases. After purification of the monoclonal immunoglobulins (both IgG_1-lambda) by isoelectric focusing [2] these were subjected to immunoelectrophoresis with anti-Fd and anti-Fab antisera (Behringwerke, Marburg, F.R.G.) and analytical ultracentrifugation. No Fd deficiency could be detected by immunoelectrophoresis, the molecular weight of monomeric immunoglobulins calculated from ultracentrifugation was approximately 160,000 daltons. These results are in accordance with amino acid analysis of the monoclonal immunoglobulin of an early described case with papular mucinosis, which did not show substantial differences from normal IgG [3]. The described Fd deficiency appears to be exceptional and may not explain the obscure skin abnormalities.

REFERENCES

1. Kitamura W, Matsuoka Y, Miyagawa S, Sakamoto K: Immunochemical analysis of the monoclonal paraprotein in scleromyxedema. J Invest Dermatol 70:305–308, 1978
2. Otavsky WI, Bell T, Saravis C, Drysdale JW: Isoelectric focusing in pevicon slabs. Anal Biochem 78:302–307, 1977
3. Lawrence DA, Tye MJ, Liss M: Isolation and characterization of the basic, diagnostic IgG globulin in the rare gammopathy, papular mucinosis. Prep Biochem 1:1–17, 1971

Peter M. Kövary, M.D.
Department of Dermatology
Reinhold E. Zimmermann, Ph.D.
Department of Physiology (II)
University of Münster
D-4400 Münster
Helmut Kerl, M.D.
Department of Dermatology
Stefan Hödl, M.D.
Department of Dermatology
University of Graz
A 8010 Graz

REPLY

We do not consider the Fd defect to be the cause of scleromyxedema although in the case reported by us demonstrated such a defect. As it is different from 2 cases reported by Kovary et al and further it possesses a different monoclonal immunoglobulin (their 2 cases demonstrate existence of monoclonal immunoglobulin at IgG_1 position while our case had monoclonal immunoglobulin at slow position), no comparison between them is possible but we would like to point out several problematic points in methods.

1. It seems that Fd can not always be separated by the purification of immunoglobulins through isoelectric focusing. It is more desirable to examine the reactions of anti-Fd, anti-Fab and anti-Fc against immunoglobulin after purification as well as the determining molecular weight using column chromatography.

2. In their 2 cases existence of stereostructural abnormality may be assumed even if normal IgG_1 and molecular weight are identical; thus it seems necessary to examine reactions with anti-Fd, anti-Fab and anti-Fc against monoclonal immunoglobulin treated with mercaptoethanol after purification.

3. Reactions with anti-Fd, anti-Fab and anti-Fc should also be examined after postpurified monoclonal immunoglobulin has been decomposed by papain.

Since we do not know the details of their methodology, it is difficult to comment further on the differences between these patients.

Dr. Y. Matsuoka, M.D.
Department Clinical Laboratory Diagnostics
Dr. W. Kitamura, M.D.
Department of Dermatology
Nara Medical University
Kashirhara Nara Japan

SUBSTANCE P

To the Editor:

Drs. Hägermark, Hökfelt, and Pernow (J Invest Dermatol 71:233–235, 1978) describe the induction of flare and itch by intradermal injection of Substance P (SP) in human skin. While these authors state that SP evoked flare, wheal, and itching at the injection site, nowhere do they describe any systemic effects of such intradermal injections. We recently injected 0.05 ml of SP intradermally into the forearms of 7 normal volunteers who had provided informed consent in concentrations ranging from 6.2×10^{-7} M to 2×10^{-9} M and were surprised by the systemic reactions we observed in all subjects. At the lowest concentrations we employed, generalized flushing was noted within 5 min of injection, and this flushing persisted for about 10 min. At higher concentrations, this generalized flushing was accompanied by extraordinary injection of the conjunctivae and sclerae, tachycardia, diaphoresis, and was followed within 10 min by generalized vasoconstriction which lasted another 10–20 min. Two subjects experienced mild wheezing and tightness in their chests, as well as uncomfortable abdominal sensations. Unfortunately, because of the unexpected nature of these systemic reactions, monitoring of cardiopulmonary status was neglected. All subjects recovered fully within 30 min and none required special medical attention or cardiovascular drugs such as epinephrine. However, all who received the highest concentration of SP employed, described their reaction as being among the most unpleasant they had experienced. Considering the exceedingly small dosages employed intradermally, one must be impressed by the potency of SP as a vasodilating agent. In light of such potent effects, inadvertant intravenous introduction of such concentrations of SP could prove fatal. We, therefore, caution other investigators who have read Hägermark et al's article to exercise extreme caution in injecting human subjects with SP.

Joel E. Bernstein, M.D.
Assistant Professor of Medicine
and Dermatology
Director, Dermato-Pharmacology Lab.
University of Chicago School of Medicine
J. Robert Hamill, Jr., M.D.
Section of Dermatology
University of Chicago School of Medicine

REPLY

Drs. Bernstein and Hamill describe systemic reactions with flushing, tachycardia, and other symptoms of vasodilation after intradermal injection of Substance P (SP). We are as surprised as they to learn about this, since we in no case observed any sign whatsoever of systemic effects after the SP injections although we gave as much as 10^{-5} M. However, we did not inject more than 0.02 ml and always in the upper arms, while Drs. Bernstein and Hamill gave 0.05 ml and in the forearms. Similar reactions as those described by them have previously been found to occur in humans during intravenous infusion of SP [1]. During infusion of 65 to 366 ng/min (5×10^{-11}–2.8×10^{-10} moles/min) the subjects described feelings of warmth and sometimes experienced temporal pulsations. At the same time a bright red flush was clearly seen in the head and neck and in isolated spots on other parts of the body [1]. Within seconds after stopping the infusions the reactions disappeared, which contrasts in a remarkable way from the observation of Drs. Bernstein and Hamill that the flushing persisted for about 10 min. They used SP from Sigma, while the SP used in our studies was synthesized by Prof. K. Folkers, Austin, Texas.

It should be added that we always perform the initial experiments

on new substances in our own skin. Recently we have injected other peptides intradermally and found vasoactive intestinal polypeptide (VIP) as potent as SP [2], but in no case any sign of systemic reactions appeared.

REFERENCES

1. Eklund B, Jogestrand T, Pernow B: Effect of substance P on resistance and capacitance vessels in the human forearm, Substance P. Edited by US von Euler, B Pernow. New York, Raven Press, 1977, pp 275–285
2. Fjellner B, Hägermark Ö: Studies on pruritogenic and histamine-releasing effects of some putative peptide neurotransmitters. Acta Dermatovener (Stockholm), in print

Östen Hägermark, M.D.
Associate Professor
Department of Dermatology
Karolinska Hospital
S-104 01 Stockholm
Sweden

BINDING OF BULLOUS PEMPHIGOID ANTIBODIES TO BASAL CELLS

To the Editor:

Takeji Nishikawa and his associates report on the co-existence of autoantibodies to the basement membrane zone and the "basal cell membrane and/or cytoplasm" in 8 of 12 sera from patients with bullous pemphigoid [1]. On the basis of this observation and some blocking immunofluorescence studies, the authors conclude that these represent 2 types of pemphigoid antibodies and that the basal cell antibodies cross react with pemphigus antibodies.

We do agree that basal cell antibodies commonly co-exist with pemphigoid antibodies. Several literature reports on the basal cell antibodies [2] indicate that they are also found occasionally in the normal human sera and that they occur more commonly following trauma to the skin; that is, in sera of patients who have suffered severe burns, graft vs. host reactions, malignancies, drug reactions, pemphigus, and pemphigoid. These findings point to a lack of disease specificity of these antibodies which stands in contrast with the basement membrane zone antibodies of pemphigoid. Another difference between the basal cell antibodies and the basement membrane zone antibodies of pemphigoid is that the latter appear to bind to *normal* skin *in vivo* [3] while the basal cell antibodies fail to do so.

Takeji Nishikawa and his associates give 2 alternate explanations for the localization of the basal cell antibodies, i.e., "basement membrane and/or cytoplasm." The picture of the indirect immunofluorescence reaction pattern of such basal cell antibodies as shown in their Figure reveals unstained spaces between some of the basal cells, thus indicating that the reactive antigen(s) occur inside the cell at the periphery of the cytoplasm. This observation favors the second of the 2 alternate interpretations by Takeji Nishikawa et al.; they do not appear to be antibodies to the membranes of basal cells. This also affords a reasonable explanation for the failure of these antibodies to bind *in vivo* to their antigen in normal skin.

Considering the 3 above-listed differences between the basal cell antibodies and the basement membrane zone antibodies of pemphigoid, (notably the differences in their disease specificity, capacity to react *in vivo* and histologic localization) we suggest an alternative explanation for the interesting findings of Takeji Nishikawa and his associates [1], notably that there are 2 distinct, unrelated antibodies which do, as the authors state, frequently co-exist with basement membrane zone antibodies. We submit further that these 2 types of autoantibodies serve as examples of two distinct types of autoimmunity [4].

Another interesting finding of these authors is that pre-treatment of frozen tissue sections with sera containing the basal cell and basement membrane zone antibodies greatly reduced the staining by FITC labeled intercellular antibodies while pre-treatment with sera containing only basement membrane zone antibodies failed to do so [1]. The authors interpret this to mean that there is some cross reactivity between pemphigus antibodies and bullous pemphigoid antibodies.

Blocking immunofluorescence studies with FITC labeled pemphigus antibodies performed in this laboratory [5] showed that some normal human sera could block the binding of labeled pemphigus antibodies just as well as sera of pemphigus patients while the IgG fractions of normal sera failed to do so. Thus, immunoglobulin fractions, not whole sera, were deemed to be acceptable for blocking IF studies. These observations raise 2 interesting questions about the blocking immunofluorescence studies of Takeji Nishikawa et al., notably: (1) if 20 or more normal sera were tested in such a blocking study, would they all be negative? and (2) if the immunoglobulin fractions of sera with basement membrane zone antibodies with and without basal cell antibodies were used for these blocking studies would they give the same results as the whole sera? The findings of Takeji Nishikawa and his associates [1] clearly merit further studies.

REFERENCES

1. Nishikawa T, Kurihara S, Harada T, Hatano H: Binding of bullous pemphigoid antibodies to basal cells. J Invest Dermatol 74:389–391, 1980
2. van Joost T: Serum antibodies with affinity to the cytoplasm of epidermal basal cells, Immunopathology of the skin, 2nd ed. Edited by EH Beutner, TP Chorzelski, and SF Bean. New York, John Wiley and Sons, 1979, 473–488
3. Binder WL: In vitro effects of pemphigus antibodies on skin. Master's Thesis, SUNY/Buffalo, 1976
4. Beutner EH, Jablonska S, Chorzelski TP, Binder WL: A unified concept of autoimmunity and the nature of SCAg conversion phenomenon, Immunopathology. Proc. of the 6th Intern. Convocation on Immunology. Edited by F Milgrom and B Albini, Karger, Basel, 1979, pp 142–147
5. Wood GW, Beutner EH: Blocking immunofluorescence studies on the specificity of pemphigus autoantibodies. Clin Immunol Immunopathol 7:168–175, 1977

Susan Krasny
Ernst H. Beutner
Vijay Kumar
Department of Microbiology
State University of New York at
Buffalo School of Medicine
Buffalo, NY 14214
Stefania Jablonska
Tadeusz P. Chorzelski
Department of Dermatology
Warsaw Academy of Medicine
Warsaw, Poland

REPLY

We are somewhat embarrassed to have the comments from Krasny et al, because the interpretation of our experimental results is completely different from the basic point [1]. Our interpretation is not the co-existence of 2 distinct antibodies but quantitative difference of reactivity by basement membrane zone (BMZ) antibodies in pemphigoid sera. As far as we can understand from the staining pattern, there is a continuity of antigenic substance from the BMZ to the lower epidermal cells, as visualized by complement immunofluorescence only.

Regarding 2 questions about the blocking immunofluorescence studies, we, too, found most undiluted normal human sera (NHS) could block the subsequent binding of FITC labeled intercellular antibodies (unpublished data). The same result could be obtained, when indirect immunofluorescent staining was applied to the skin substrate treated with undiluted NHS. However, NHS at 1:10 or more dilutions did not give any blocking of the subsequent staining of intercellular antibodies. We interprete this phenomenon as the direct effect of undiluted NHS [2] or the buffer [3] to the labile intercellular antigen(s) of the epidermis. We do not believe this experience of intercellular substance is applicable to the case of BMZ, since the antigenic property of intercellular substance and BMZ is largely different. As shown in the Table III of our paper [1], the serum dilution of BP sera and that of the control NHS was 1:10 dilution, where no such blocking effect of the serum to the skin substrate was shown.

We obtained the most purified IgG fraction from T.H. serum [1] by $(NH_4)_2SO_4$ treatment and G-200 column chromatography and found that this purified IgG at 7 units could block the subsequent binding of FITC labeled T.Y. at 2 units. Although more sera should be tested to draw the definite conclusion, it seems as if our findings could be justified.

REFERENCES

1. Nishikawa T, Kurihara S, Harada T, Hatano H: Binding of bullous pemphigoid antibodies to basal cells. J Invest Dermatol 74:389–391, 1980
2. Nishikawa T, Kurihara S, Harada T, Sugawara M, Hatano H: Capability of complement fixation of pemphigus antibodies in vitro. Arch Dermatol Res 260:1–6, 1977
3. Ogawa H, Taneda A, Morioka S: Characterization of pemphigus antigen. J Invest Dermatol 70:194–196, 1978

Takeji Nishikawa
Seiichi Kurihara
Takashi Harada
Hitoshi Hatano
Department of Dermatology
Keio University School of Medicine
Tokyo